Novel Trafficking Assays for Pharmacochaperone Discovery Using Enzyme Fragment Complementation

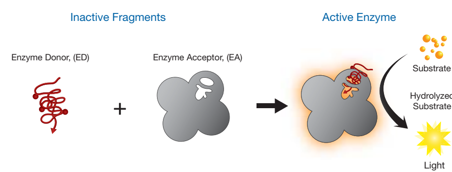
Daniel Bassoni, Qumber Jafri, Mong Saetern, Philip Achacoso, Dana Haley-Vicente, Ph.D., and Jane Lamerdin, Ph.D.

DiscoveRx Corporation, Fremont, CA 94538, USA

Abstract

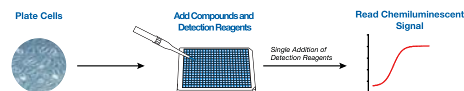
DiscoveRx® has pioneered a novel enzyme complementation system to monitor cellular events such as protein translocation, protein interactions, and degradation in an HTS friendly, cell-based format. This unique technology provides a basis for the generation of novel cellular assays for intractable targets, and simplify the detection of cellular signaling events using a simple one-step chemiluminescent protocol. We have applied this technology towards the development of cell-based assays designed to detect pharmacochaperone mediated trafficking of proteins with mutations that otherwise degrade normal trafficking to the cell membrane. Disease relevant ion channel and GPCR trafficking mutants linked to cystic fibrosis, retinitis pigmentosa, severe early-onset obesity, basal cell carcinoma and nephrogenic diabetes insipidus will be highlighted.

Enzyme Fragment Complementation Technology



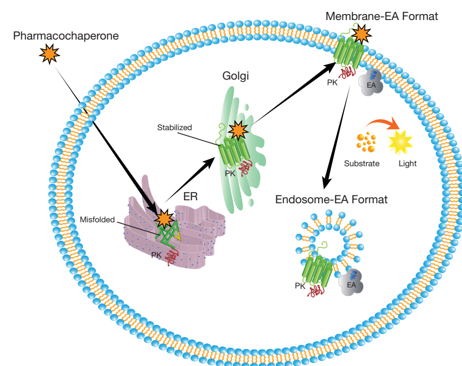
DiscoveRx has pioneered a well established technology called Enzyme Fragment Complementation (EFC). This robust technology is based on the complementation of two inactive β-galactosidase enzyme fragments, EA (enzyme donor) which encodes the majority of the enzyme, and a small peptide or ED (enzyme acceptor) termed ProLink™. The ProLink tag can be used to tag proteins and monitor their secretion, degradation, internalization, trafficking and other cellular processes in cells following the addition of the recombinant EA fragment localized in the cell and PathHunter® substrate.

Protocol and Detection



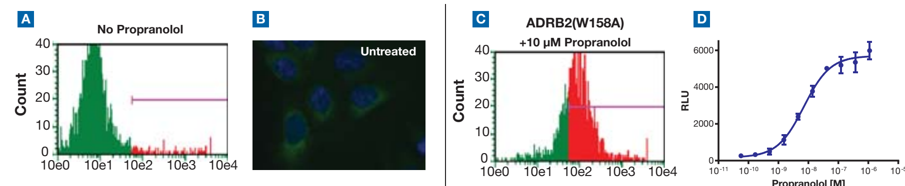
The cells are seeded in 384-well plates and incubated in assay media. Cells are then treated with the compound. This is followed by the addition of PathHunter Detection reagents which are added as a single mix-and-read reagent and includes a lysis buffer and substrate mixture optimized for enzyme complementation. After a short incubation, the PathHunter chemiluminescent signal can be detected using any standard luminometric plate reader.

PathHunter Pharmacotrafficking Assay



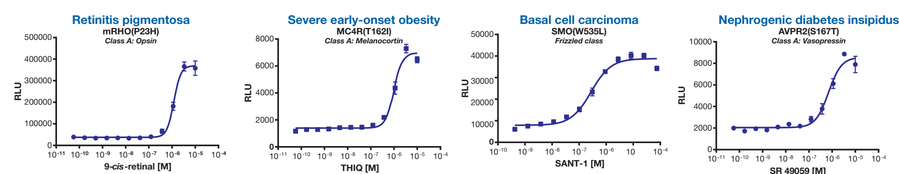
In the PathHunter Pharmacotrafficking Assay, the EA is localized to a specific subcellular compartment (either at the membrane or on the early endosome) and the protein of interest is tagged with ProLink (PK). An increase in concentration of the ProLink tag in the vicinity of EA (i.e. target-PK/Membrane-EA or target-PK/Endosome-EA) results in complementation of the enzyme fragments. Upon addition of substrate, this complementation results in a measurable chemiluminescent signal. In this manner, the localization of the protein fused to ProLink dictates the amount of complementation and signal to be detected.

β2-Adrenoceptor Mutant ADRB2 (W158A)



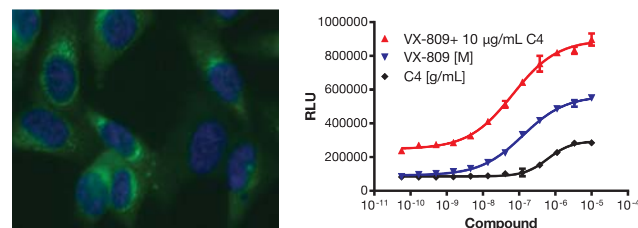
A mutant form of the class A GPCR, β2-Adrenoceptor (ADRB2) containing a single engineered point mutation (W158A) was analyzed. Wildtype ADRB2 in its inactive state localizes to membrane, while the mutant form of the receptor is misfolded and retained in the ER. When the cells are untreated with the pharmacochaperone propranolol, FACS (**A**) and immunofluorescent imaging (**B**) experiments show the receptor is not localized to the membrane. When cells are treated with propranolol, analysis using FACS (**C**) and PathHunter Pharmacotrafficking Assay (**D**) both indicate the receptor has been rescued and has trafficked to the membrane. Overall, the small molecule pharmacochaperone was able to induce the redistribution of mutant ADRB2 from ER to membrane.

Disease Relevant GPCR Trafficking Mutants



Examples of disease relevant mutant GPCRs using various small molecule pharmacochaperones to stabilize the receptors and allow for their proper trafficking to the membrane and detection as analyzed the PathHunter Pharmacotrafficking Assay.

PathHunter CFTR-ΔF508 Pharmacotrafficking



Analysis of a mutant form of the ion channel cystic fibrosis transmembrane conductance regulator (CFTR) containing a single point deletion CFTR-ΔF508 was conducted. This deletion is the most common mutation in cystic fibrosis patients; it causes the protein to misfold, thus preventing efficient trafficking and leading to ER retention (immunofluorescence image, left). Testing the PathHunter CFTR-ΔF508 Pharmacotrafficking assay with a combination of two pharmacochaperone compounds, C4 and VX-809, stabilizes the mutant receptor, allowing for proper trafficking. The dual treatment results in elevated signal all along the curve (right, red curve) indicating an additive effect, which is the expected behavior of the combination of the two compounds.

Summary & Conclusions

Here we demonstrate the implementation of the Enzyme Fragmentation Complementation technology for studying pharmacochaperone mediated forward trafficking of transmembrane proteins with trafficking defects. The PathHunter Pharmaco- trafficking assay is engineered to detect the trafficking of a ProLink-tagged mutant protein from the ER to the plasma membrane or early endosomes. This novel cell-based assay is high-throughput, easy-to-use, and robust, allowing for monitoring forward trafficking of ER-retained mutant targets. These results indicate the system provides a powerful method for screening small molecule libraries to discover pharmacochaperones of disease relevant or orphan GPCRs, ion channels, and transporters.

tel | 1.866.448.4864 tel | +44.121.260.6142 e | info@discoverx.com

© 2015 DiscoveRx Corporation. All Rights Reserved. 20300 010515

DiscoveRx
www.discoverx.com